

Methods for determination of particle size distribution —

Part 4: Guide to microscope and image analysis methods

Committees responsible for this British Standard

The preparation of this British Standard was entrusted by the General Mechanical Engineering Standards Policy Committee (GME/-) to Technical Committee GME/29, upon which the following bodies were represented:

- BCIRA
- British Cement Association
- British Coal Corporation
- British Laboratory Ware Association
- Coated Abrasives Manufacturers' Association
- Guild of Metal Perforators
- Institution of Chemical Engineers
- Institution of Mining and Metallurgy
- Mechanical Handling Engineers Association
- Ministry of Defence
- NABIM
- Society of Chemical Industry
- Society of Cosmetic Scientists
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- Aluminium Federation
- China Clay Association
- Department of Trade and Industry (National Physical Laboratory)
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- Health and Safety Executive
- Royal Pharmaceutical Society of Great Britain
- Royal Society of Chemistry
- Sunderland Polytechnic (Magnet Centre)
- University of Bradford
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Foreword

This revision of BS 3406-4 has been prepared under the direction of the General Mechanical Engineering Standards Policy Committee and supersedes BS 3406-4:1963 which is withdrawn.

It is one of a series of methods for determining the size distribution of particles.

Other Parts of BS 3406 are:

- *Part 1: Guide to powder sampling;*
- *Part 2: Recommendations for gravitational liquid sedimentation methods for powders and suspensions;*
- *Part 3: Air elutriation methods (obsolescent);*
- *Part 5: Recommendations for electrical sensing zone method (the Coulter principle);*
- *Part 6: Recommendations for centrifugal liquid sedimentation methods for powders and suspensions;*
- *Part 7: Recommendations for single particle light interaction methods.*

The particle size distribution of a powder is an important basic property that can distinguish it from other samples of the same composition. Vast quantities of powdered materials are sold or processed and specifications often include average particle size and/or particle size distribution. Even when other bulk properties are specified, compliance with the specification may be achieved by varying the particle size distribution. Particle size analysis is also used extensively to monitor environmental conditions and in numerous areas of scientific research. In all these contexts the reproducibility and comparability of results is of paramount importance and the use of standardized techniques is essential.

Representative sampling and correct dispersion of particulate material are prerequisites for the determination of size distribution. The procedures described in BS 3406-1 have been selected to give test portions representative of the bulk.

The techniques used to measure particle size distribution are many and are varied both in principle and in their degree of technical complexity and automation. In principle, any property that depends on particle size can be used as a means of measuring particle size. In practice, the methods that are widely used are both experimentally convenient and based upon a physical principle having a well defined relationship to particle size.

The choice of the most suitable method will depend on the following considerations:

- a) the purpose for which the analysis is required, e.g. quality control, research, specification requirements;
- b) the size range and other properties of the particles, e.g. density, solubility, refractive index;
- c) aggregation and dispersion characteristics of the powder;
- d) the amount of material available for analysis;
- e) the method by which the sample has been collected;
- f) the conditions under which the powder is to be used, for example the state of dispersion in the test should be related to the state of dispersion in the application;
- g) the resources available.

Microscopy is the only method in which direct observation is made of the size and shape of the particles. Most methods of particle size measurement ascribe a single dimension to each particle, usually expressed as the diameter of the spherical particle equivalent to some aspect of its properties, e.g. volume, surface, settling velocity, light scattering. Physical properties of the particles, such as solubility, friability, refractive index and conductivity, preclude the use of some methods. Nevertheless the same particles can be analysed using a range of techniques and if different size parameters are determined, different size distributions will result. The intercomparison of results is meaningful only when the same parameters of a representative sample are measured using reliable equipment and standard operating procedures. Standardization between laboratories is best established by the use of certified reference materials, for example those from the Community Bureau of Reference (BCR).

Microscopy is a widely used method of particle size analysis. One of its main benefits is that it allows the characterization of the shape of particles and the detection of agglomerates and contaminants. A sample being prepared for analysis by a non-microscope, automatic method should be examined under the microscope to ensure that it is suitable and well enough prepared. Microscope measurements are usually expressed as the diameter of a sphere of equivalent projected area. The first edition of BS 3406-4 in 1963 described the use of an eyepiece graticule with a series of circular spots drawn on it, against which the images of the particles were compared and classified. The only microscope covered by the standard was the light microscope fitted with a graduated draw tube. Much has changed since 1963; the electron microscope with its high resolution and great depth of field is routinely used, the image analyser has taken much of the effort out of taking measurements and the desktop computer allows complicated data analysis to be carried out on large amounts of data. This revision of the standard reflects these changes although many of the principles remain unaltered.

Microscopy is an apparently simple method of particle size analysis but in practice great care is needed if accurate results are to be achieved. The quality of the final result will suffer from the addition of uncertainties introduced at all stages of the method from sample preparation through to data analysis.

It has been assumed in the drafting of this British Standard that the execution of its provisions is entrusted to appropriately qualified and experienced people.

It is expected that users of this standard will be conversant with the appropriate measuring instrument manufacturer's instruction manual.

WARNING. This British Standard may involve the use of substances and/or procedures that may be injurious to health if adequate precautions are not taken. It refers only to technical suitability and does not absolve the user from legal obligations relating to health and safety at any stage. Attention is drawn to the specific hazards described in clause 5.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations. In particular, attention is drawn to Health and Safety Executive Control of Substances Hazardous to Health Regulations (1988) (COSHH).

Summary of pages

This document comprises a front cover, an inside front cover, pages i to iv, pages 1 to 32, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

Introduction

This standard covers the measurement of particle size by light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Light microscopy is suitable for the measurement of particles in the size range from 3 μm to 1 mm, SEM from 20 nm to 1 mm and TEM from 2 nm to 1 μm . Microscopy uses a very small test portion. This has advantages if the laboratory sample is itself small or if the particles are expensive. The disadvantage with a small test portion is that even greater care is needed to produce a representative sample. Microscopy is particularly suited to making measurements on particles that are collected on a filter by a sampler if the filter can then be placed directly under the microscope.

Image analysis (IA) has removed a considerable amount of the hard work involved in making manual measurements. The IA will make measurements all day, the last one being as good as the first. The IA introduces its own uncertainties into measurements. In the same way that samples for size analysis by methods other than microscopy should be looked at with a microscope first, so too, test portions for automatic microscope analysis should be examined by eye before starting measurements. IAs are often equipped with the facility to control focus, delineation and image brightness automatically. These are useful functions but they have to be used with care because they can have unwanted effects if presented with an unusual image. A good understanding of the IA and its limitations is needed.

In this standard the particle size is expressed as the equivalent circle diameter. Except in the case of spherical particles the size distribution arrived at is unique to microscopy and cannot be related directly to the results of other sizing techniques.

This standard includes methods of calculating the mean and standard deviation of the particle size distribution from the measured data. The use of IAs to make the measurements, and microcomputers to calculate the statistics, means that large amounts of data can be generated and processed quickly. The use of many measurements reduces the effect of random uncertainties but has no effect on systematic uncertainties. It is sensible to make some measurements on particles, or other reference objects, of known size so that the likely systematic uncertainties introduced by the equipment can be calculated. This enables a realistically required confidence interval to be estimated.

Despite all the innovations in microscope methods, the fundamentals remain the same. There is no substitute for representative sampling and good microscopy.

1 Scope

This Part of BS 3406 recommends methods for using microscopes to measure the size of particles in the range from 2 nm to 1 mm. Making measurements with light microscopes, scanning electron microscopes and transmission electron microscopes is described both with and without the use of automatic and semi-automatic image analysers. A system is established for calculating the number of fields of view that have to be analysed to achieve a specified accuracy, and methods are given for the estimation of uncertainties. Guidance is provided on sample preparation, practical microscopy and the choice of equipment for image analysis.

This Part of BS 3406 does not cover the measurement of particle shape or the use of stereological methods.

This standard does not cover the measurement of asbestos fibres. Asbestos fibres are covered by the European Reference Method (ERM) as given in MDHS 39¹⁾.

Specialized techniques applicable to the study of cell biology or haematology are outside the scope of this Part of BS 3406.

2 References

2.1 Normative references

This Part of BS 3406 incorporates, by reference, provisions from specific editions of other publications. These normative references are cited at the appropriate points in the text and the publications are listed on the inside back cover. Subsequent amendments to, or revisions of, any of these publications apply to this Part of BS 3406 only when incorporated in it by updating or revision.

¹⁾ Available from the Health and Safety Executive.

2.2 Informative references

This Part of BS 3406 refers to other publications that provide information or guidance. Editions of these publications current at the time of issue of this standard are listed on the inside back cover, but reference should be made to the latest editions.

3 Definitions

For the purposes of this standard, the definitions given in BS 2955:1958 apply, together with the following.

3.1

auto-delineation

the process which attempts to predict the true edge of unsharp images of particles by reference to the local grey level values. This is used to improve the accuracy of measurements and to make them less sensitive to incorrect grey level thresholding

3.2

binary image

a digitized image consisting of an array of pixels, each of which has a value of either 0 or 1. These values are normally represented by dark and bright regions on the display screen or by the use of two distinct colours

3.3

detection

the process by which the digitized grey level image is converted into a binary image representing the particles to be measured. The simplest form of detection involves setting a grey level value above which a pixel will be counted as being part of the particle; all other pixels will then be counted as belonging to the background

NOTE Segmentation and thresholding are commonly found synonyms for detection.

3.4

digitization

the process of converting the continuously varying voltage output of a video signal into a discrete array of pixels whose values correspond to the intensities of the parts of the original image that they represent

3.5

equivalent circle diameter

the diameter of a circle having the same area as the project image of the particle

3.6

image frame

the total area of the specimen displayed on the monitor at a given time

3.7

measurement frame

a subframe within the image frame over which the measurements are made

3.8

mouse

a device to allow interaction, by means of the operator's hand, with the computer

3.9

numerical aperture

product of the refractive index of the object space and the sine of the semi-aperture of the cone of rays entering the entrance pupil of the objective lens from the object point

NOTE The resolving power is proportional to the numerical aperture.

4 Abbreviations and symbols

4.1 Abbreviations

For the purposes of this standard, the following abbreviations apply.

BCR	Community Bureau of Reference
ECD	Equivalent circle diameter
IA	Image analysis/analyser
NA	Numerical aperture
SEM	Scanning electron microscope/microscopy
TEM	Transmission electron microscope/microscopy

4.2 Symbols

For the purposes of this standard, the following symbols apply.

Symbol	Quantity
A_r	Total area of measurement of r^{th} class
a_r	Field of view for one measurement
B	Average number of particles per unit area
c	Integer used to calculate class interval
d_r	Geometric mid-class ECD
$E()$	Expectation error of ()
i	Integer
j	Number of classes
k_i	Constant dependent on particle shape and density
m_r	Number of particles in the r^{th} class
M_i	Expectation error of m_i [$= E(m_i)$]
n_r	Number of fields of view of r^{th} class
N	Total number of particles measured
P_i	Expectation error of p_i [$= E(p_i)$]
P_r	Number size proportion (100 P_r = percentage number in r^{th} class)
Q_i	Expectation error of q_i [$= E(q_i)$]
q_r	Volume size proportion (100 q_r = percentage number in r^{th} class)
r	Size class number
$S()$	Standard error in ()
T	Particle thickness
t	Number proportion
u_i	Mean of x_i
u	Sum of all u_i
V_i	Variance of x_i
V	Sum of all V_i
$\text{var}()$	Variance of ()
w_i	Confidence interval width for the i^{th} particle
$\times$	Magnification, e.g. $\times 40$
x_i	Independent random variable
Y_{app}	Apparent length of eyepiece graticule
Y_{ref}	Reference length of required graticule
Y_{req}	Required apparent length of eyepiece graticule
Y_{true}	True length of eyepiece graticule

Symbol	Quantity
z_i	$x_i - u_i$
z	Sum of all z_i
α	Angular aperture of SEM probe
β	Expected number of particles per unit area
θ	Half angle subtended by particle at objective
λ	Wavelength
μ	Reference index of the surrounding medium
ψ	Desired percentage accuracy for i^{th} particle
φ	Phase shift

5 Health and safety

Good laboratory practice should apply to the use of chemicals and in the preparation of samples, especially toxic dusts. To prevent inhalation, these materials should be handled in a suitable fume cupboard or glove box.

6 Sample preparation

6.1 General

Samples should be subdivided in accordance with BS 3406-1:1986. There are only general rules for sample preparation for the microscope, and no specific rule will cover every possible situation. It is important always to remember the following.

- The energy of dispersion during sample preparation in the laboratory should be as closely equivalent as possible to the energy of dispersion in product use. This is particularly important for agglomerates, which may behave as single particles, and for fragile particles.
- Microscopical inspection is recommended to assess the degree of dispersion that has been achieved prior to image analysis.
- It should be accepted that it is not possible to prepare perfect samples routinely and repetitively. A procedure should be devised to ensure maximum reproducibility. Generally, the simpler the procedure, the smaller the error, and hence the greater the reproducibility. When the mounting technique is chosen, it should be rigidly adhered to so that no bias exists between samples.
- In circumstances where a mounting medium is used, ideally it should be chosen to give good image contrast between the particles and that medium. It should not react or dissolve the particles. Regular blank checks for cleanliness should be performed.
- The mounting surfaces should always be clean before use. For example, high quality slides and coverslips should be used and should be degreased with solvent and dried with a jet of filtered air.
- It is essential that the test portion is not excessively loaded with particles since particle contact or overlap will give misleading results. Partial compensation for overlap can be gained using adjustments of focus in optical microscopy or of the depth of field in scanning electron microscopy. It is important to note that particles which apparently touch on the image analysis screen will, unless separated, be treated as a single particle. Some image analysis systems implement manual or automatic de-agglomeration routines that can be used to separate particles, but overlapping particles would have an apparent size that is less than the true size and this will inevitably produce misleading results.

6.2 Dispersion of laboratory sample

Sampling from liquids is generally the easiest method, providing the laboratory has a suitable piece of equipment for uniform dispersion. Providing the criteria listed in 6.1 apply, the simplest way is to agitate and stir the suspension in a low power ultrasonic bath and to extract suitable aliquots. For less robust particles or agglomerates, an alternative method is shown in Figure 1 in which an air jet circulates the suspension through a sampling tube which can be closed and withdrawn to provide samples for analysis. The microscopist has a choice of suspending the material on a filter [see 6.3 a)], using a straight-sided cavity slide (see Figure 2) to receive an aliquot [see 6.3 b)], using a rod to drop the suspension onto a microscope slide [see 6.3 c)], or smearing a paste [see 6.3 d)].

For sub-micrometric particles see the SEM method given in 6.3 a).

6.3 Mounting of test portion

The test portion should be mounted by one of the following methods.

a) *Mounting by filtration*

NOTE 1 This method is suitable for light microscopy scanning electron microscopy and transmission electron microscopy. If the filter, or its support, is not completely dry before commencing the filtration, the particles will not be evenly distributed. Large-holed filter supports should be avoided or their effects minimized by the use of backing pads to ensure an even distribution of particles. It should be recognized that, if the particles are irregularly shaped, they will adopt preferred orientation on their largest face. The speed of filtration should be such that preferential settling of particles is avoided. Pressure filtration is quicker than vacuum filtration as a pressure of several atmospheres can be used. The pore size of the filter should be suitable for the collection of the particles to be sized and should not result in loss of particles or deep penetration of particles into the filter.

For light microscopy, the particles can be assessed by reflected light or, if the filter can be made transparent by a suitable process, by transmitted light. The clearing process should not involve the movement of particles on the filter surface. There should be good optical contrast between the particle and the surrounding medium.

For scanning electron microscopy, a smooth surfaced filter such as a polycarbonate membrane filter should be chosen. A suitable conducting film (e.g. gold or carbon) should be coated onto the preparation. For analysis by transmission electron microscopy, the filter is prepared for examination by plasma ashing, carbon coating and solvent dissolution. The process is not suitable for organic particles.

b) *Mounting by straight-sided cavity slide*

NOTE 2 This method is suitable for light microscopy.

The refractive index of the mounting medium should be very different from that of the particles to ensure good optical contrast. Care should be taken to ensure that there is no preferential loss of a size class when the coverslip is added. The density of the liquid should be less than the density of the particles to encourage the particles to lie in one plane of focus at the bottom of the preparation, but this does not aid distribution in the initial mixing. Large particles will settle more quickly than small particles. The technique can therefore involve considerable changes of focus during analysis to ensure that all of the particles are sized. If the liquid is subject to viscosity variations with temperature, this will influence the settling rate. The settling of particles will also involve preferential orientation.

c) *Mounting by a drop of suspension on a microscope slide*

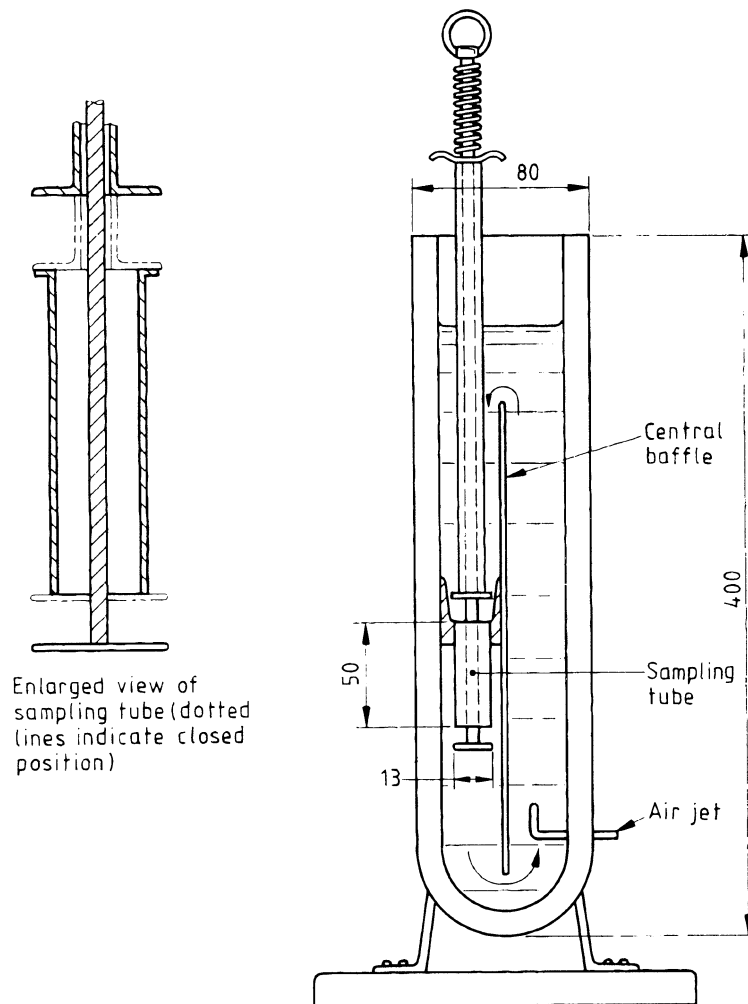
NOTE 3 This method is suitable for light microscopy with image analysis.

The refractive index of the mounting liquid should be very different from that of the particles. The drop should be centrally covered with a circular coverslip. This technique will always give a radial distribution of particle numbers and particle size. If it is ever used, it is important to perform the analysis of sectors to help compensate for this, and several preparations should be examined to ensure reproducibility, rather than accepting the results of one or two test portions. The technique can work quite well for automatic particle sizing when it is possible to assess large areas of the test portions rapidly.

d) *Mounting in a paste*

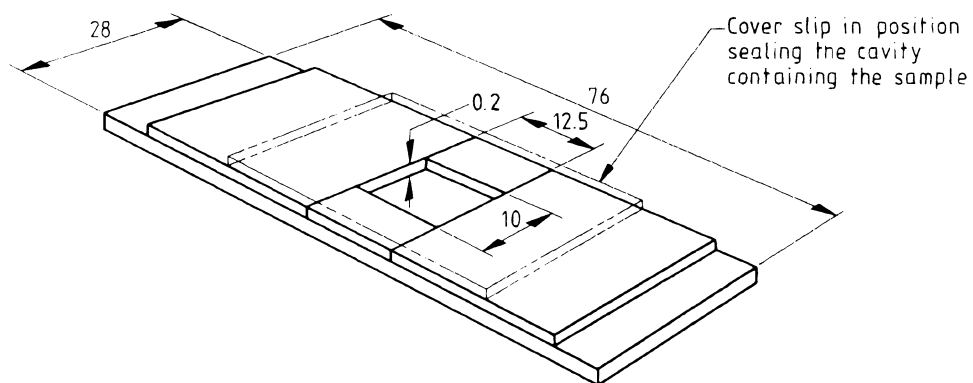
NOTE 4 This method is suitable for light microscopy.

The refractive indices of the liquid and the particles should be very different to ensure good optical contrast. When the paste is placed on the slide, it should be covered with a centrally placed circular coverslip and sectors should be surveyed to compensate for the radial distribution.



All dimensions are in millimetres.

Figure 1 — Liquid sample divider



All dimensions are in millimetres.

Figure 2 — Typical cell for examination of particles dispersed in liquid of low viscosity